

WE CLAIM:

1. A method of producing a population from neural tissue enriched for human central nervous system stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC), comprising:
  - 5 a) contacting neural or neural derived cells with a monoclonal antibody that binds to CD49f; and
  - b) selecting said neural or neural-derived cells that bind to the monoclonal antibody; wherein the selected cells are enriched for human CNS-SC.
- 10 2. The method of claim 1, wherein the monoclonal antibody is fluorochrome conjugated.
3. The method of claim 1, wherein the monoclonal antibody is conjugated to magnetic particles.
- 15 4. The method of claim 1, wherein the selecting is by flow cytometry (fluorescence activated cell sorting) or high gradient magnetic selection.
5. The method of claim 1, wherein the population containing neural or neural-derived cells is obtained from a neurosphere culture or an adherent culture.
- 20 6. The method of claim 1, wherein the population containing neural or neural-derived cells is obtained from primary neural tissue.
- 25 7. The method of claim 1, further comprising the steps of further enriching a population from neural tissue for CNS-SC by
  - c) contacting the selected cells with a second monoclonal antibody SC20, recognizing the CD24 antigen; and
  - d) removing those cells that are SC20<sup>+</sup> (CD24<sup>+</sup>),

30 wherein the selected cells in the population are SC20<sup>-lo</sup> (CD24<sup>-lo</sup>) and are enriched for CNS-SC.

8. The method of claim 1, further comprising the steps of further enriching a population for CNS-SC by

5           c) contacting the selected cells with an anti-CD133 monoclonal antibody; and

          d) further selecting those cells that bind to the anti-CD133 monoclonal antibody, wherein the further selected cells are enriched for CNS-SC.

9. The method of claim 1, wherein the neural or neural derived cells are contacted with an anti-CD133 monoclonal antibody prior to the contacting of step a).

10       10. A method for producing a population enriched for human central nervous system stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC) or an adherent cell culture comprising selecting from a population of neural or neural-derived cells for those cells that are CD49f<sup>+</sup>.

15       11. The method of claim 10, wherein said selection is accomplished by contacting the population of cells with an anti-CD49f antibody selected from the group consisting of monoclonal antibody GoH3 and monoclonal antibody 4F10 and removing those cells that do not bind to the anti-CD49f antibody.

20       12. The method of claim 11, further comprising the step of further enriching the population obtained from primary neural tissues for CNS-SC by selecting the cells that are CD24<sup>-lo</sup> from the remaining population of neural or neural-derived cells.

25       13. The method of claim 12, further comprising the step of further enriching the population by removing the cells that bind to monoclonal antibody SC20, which recognizes CD24.

30       14. A method for enriching from a population of neural cells for the populations of neurosphere initiating stem cell (NS-IC) fraction, comprising selecting from the neural cells for cells that express CD49f by binding to monoclonal antibody GoH3 or monoclonal antibody 4F10, wherein the selected cells are enriched in the fraction of NS-IC as compared with the population of neural cells.

15. The method of claim 14, further comprising the step of further enriching for the fraction of NS-IC from primary tissues by removing the cells that are CD24<sup>+</sup>, wherein the remaining cells are CD24<sup>-lo</sup>.

5 16. A method for isolating a neurosphere initiating stem cell (NS-IC) from primary neural tissues, comprising:

- a) selecting from a population of neural or neural-derived cells for cells that are CD49f<sup>+</sup>;
- b) removing the cells that are CD24<sup>+</sup>, wherein the remaining cells are CD24<sup>-lo</sup>;
- 10 c) introducing the cells remaining after step b) to a serum-free culture medium containing one or more growth factors selected from the group consisting of leukemia inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF; FGF-2), and combinations thereof; and
- d) proliferating the remaining cells in the culture medium.

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17. A method for producing a population enriched for human central nervous system stem cells (CNS-SC) which can initiate neurospheres (NS-IC) comprising selecting from neural or neural-derived cells for cells that are CD49f<sup>+</sup> and bind to an anti-CD49f antibody selected from the group consisting of monoclonal antibody GoH3 and

20 monoclonal antibody 4F10, to produce a population enriched for CNS-SC, wherein the selecting is by attachment to and disattachment from solid phase.

18. An antibody that specifically binds to the CD49f antigen, wherein said CD49f antigen specifically binds to the monoclonal antibody GoH3 or to the monoclonal antibody 4F10.

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19. The antibody of claim 18, wherein said antibody is a monoclonal antibody produced by a hybridoma cell line.

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20. The antibody according to claim 19, wherein said monoclonal antibody blocks simultaneous binding to said CD49f antigen by the GoH3 antibody or the 4F10 antibody.

21. A method for the enrichment of human central nervous system neural stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC), said method comprising:

- combining a population of neural or neural-derived cells with a reagent that specifically binds to the CD49f antigen; and
- selecting for those cells that bind to the reagent,

wherein the selected cells are enriched for CNS-SC, progenitors, or a combination thereof.

10 22. The method according to claim 21, wherein said reagent is at least one antibody.

23. A method for producing a population enriched for human central nervous system stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC), comprising selecting from neural or neural-derived cells for those cells that express CD49f and bind to monoclonal antibody GoH3 or to monoclonal antibody 4F10, to produce a population enriched for CNS-SC, progenitors, or a combination thereof.

15 24. The method of claim 23, wherein the antibody is monoclonal antibody GoH3.

20 25. The method of claim 23, wherein the antibody is monoclonal antibody 4F10.

26. The method of claim 23, wherein the population containing neural or neural-derived cells is obtained from a neurosphere culture or an adherent monolayer culture.

25 27. The method of claim 23, wherein the population containing neural or neural-derived cells is obtained from neural tissue.

28. A method for producing a population enriched for human central nervous system stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC), wherein the population is obtained from primary neural tissues, the method comprising selecting from a population of neural or neural-derived cells for cells that are CD49f+, the method further comprising the steps of further enriching for CNS-SC,

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progenitors, or a combination thereof, by further selecting for those cells that are CD24<sup>-/lo</sup>.

29. A method for isolating a neurosphere initiating stem cell (NS-IC), comprising:
  - 5 a) selecting from a population of neural or neural-derived cells for at least one selected cell that binds to monoclonal antibody GoH3 or to monoclonal antibody 4F10;
  - b) introducing at least one selected cell to a serum free culture medium containing one or more growth factors selected from the group consisting of leukemia inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2) and combinations thereof; and
  - 10 c) proliferating the at least one selected cell in the culture medium.
30. A method of producing further subdivided populations enriched for human central nervous system stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC), comprising:
  - 15 a) contacting neural or neural derived cells with a monoclonal antibody that binds to CD15; and
  - b) selecting said neural or neural-derived cells that are CD15<sup>-/lo</sup> or CD15<sup>+</sup>; wherein the selected cells are enriched for human CNS-SC, progenitors, or a combination thereof and wherein the CD15<sup>-/lo</sup> cells are a subset of the CD133<sup>+</sup>CD24<sup>-/lo</sup> population.
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31. The method of claim 30, wherein the population containing neural or neural-derived cells is obtained from primary neural tissue.
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32. The method of claim 30, further comprising the steps of further enriching a population for CNS-SC, progenitors, or a combination thereof, by
  - c) contacting the selected cells with a second monoclonal antibody SC20; and
  - d) further selecting for those cells that are SC20<sup>-/lo</sup>, wherein the further selected cells in the population are enriched for CNS-SC, progenitors, or a combination thereof.
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33. The method of claim 30, further comprising the steps of further enriching a population for CNS-SC, progenitors, or a combination thereof, by

5           c)     contacting the selected cells with an anti-CD133 monoclonal antibody; and

          d)     further selecting those cells that bind to the anti-CD133 monoclonal antibody, wherein the further selected cells are enriched for CNS-SC, progenitors, or a combination thereof.

10           34. The method of claim 30, wherein said selection is accomplished by contacting the population of cells with monoclonal antibody MMA, recognizing the CD15 antigen, and removing those cells that do not bind to monoclonal antibody MMA.

15           35. An antibody that specifically binds to the CD15 antigen, wherein said CD15 antigen specifically binds to the monoclonal antibody MMA and wherein said antibody is a monoclonal antibody produced by a hybridoma cell line.

20           36. The antibody according to claim 35, wherein said monoclonal antibody blocks simultaneous binding to said antigen by the MMA monoclonal antibody.

          37. The method of claim 34, further comprising the step of further enriching the population for CNS-SC by removing the cells that are CD24<sup>+</sup> from the remaining population of neural or neural-derived cells, wherein the cells are obtained from primary neural tissues.

25           38. The method of claim 37, further comprising the step of further enriching the population by removing the cells that are CD24<sup>+</sup>, wherein the remaining cells are CD24<sup>-/lo</sup>.

          39. A method for enriching from a population of neural cells for the populations of neurosphere initiating stem cell (NS-IC) fraction, comprising selecting from the neural cells for cells that bind to monoclonal antibody MMA, wherein the selected cells are enriched in the fraction of NS-IC as compared with the population of neural cells.

40. The method of claim 39, further comprising the step of further enriching for the fraction of NS-IC by removing the cells that are SC20<sup>+</sup>, wherein the remaining cells are SC20<sup>-/lo</sup> and wherein the cells are obtained from primary neural tissues.

5 41. A method for isolating a neurosphere initiating stem cell (NS-IC), comprising:

- a) selecting from a population of neural or neural-derived cells for cells that are CD15<sup>+</sup> or CD15<sup>-/lo</sup>, wherein the CD15<sup>-/lo</sup> cells are a subset of the CD133<sup>+</sup>CD24<sup>-/lo</sup> population;
- b) removing the cells that are SC20<sup>+</sup>, wherein the remaining cells are SC20<sup>-/lo</sup>;
- 10 c) introducing the remaining cells to a serum-free culture medium containing one or more growth factors selected from the group consisting of leukemia inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF; FGF-2), and combinations thereof; and
- d) proliferating the remaining cells in the culture medium.

15 42. A method for the enrichment of human central nervous system neural stem cells (CNS-SC), progenitors, or a combination thereof, which can initiate neurospheres (NS-IC), said method comprising:

20 a) combining a population of neural or neural-derived cells with a reagent that specifically binds to the CD15 antigen; and

b) selecting for those cells that are CD15<sup>+</sup> or CD15<sup>-/lo</sup>, wherein the CD15<sup>-/lo</sup> cells are a subset of the CD133<sup>+</sup>CD24<sup>-/lo</sup>,  
wherein the selected cells are enriched for CNS-SC, progenitors, or a combination thereof.

25 43. The method according to claim 42, wherein said reagent is at least one antibody.